



## Characterization of Virulence and Diversity of *Puccinia graminis* f. sp. *tritici* on Wheat in Egypt

Doaa R. El-Naggar, Walid M. El-Orabey, Mohamed A. Gad<sup>#</sup>, Gamalat A. Hermas  
Wheat Diseases Research Department, Plant Pathology Research Institute,  
Agricultural Research Center, Giza 12619, Egypt.



STEM rust caused by *Puccinia graminis* f. sp. *tritici* is a destructive disease of wheat in Egypt and worldwide. Survey of wheat stem rust samples and identification of physiological races using twenty single *Sr* genes are very important in describing virulence pattern variation, the geographical distribution of stem rust pathotypes, and how its change in response to host selection. Variability in the population of the causal organism was determined using samples collected from wheat-growing areas in Egypt for two growing seasons, that is, 2015/2016 and 2016/2017. The results obtained showed significant variability in pathotypes, which are different from season to season. In the course of this study, a total of 104 and 40 stem rust samples were collected in 2015/2016 and 2016/2017, respectively from different wheat-growing areas in six governorates of Egypt, that is, Beheira, Kafr-Elsheikh, Sharqiya, Minufiya, Bani Sweif, and Sohag. A total of 70 and 53 physiologic races were isolated from samples collected in the previous growing seasons and identified in 2016/2017 and 2017/2018, respectively. The most frequent races included TKTTC (18.25%) and TTTTC (17.46%) in 2016/2017 as well as PKSTC (6.25%), BBBBC (4.69%) and PKSTH (4.69%) in 2017/2018. For 2016/2017, 24 pathotypes were identified in Kafr-Elsheikh, which consider the largest population size (34.28%), while for 2017/2018, the Sharqiya governorate considered to be the largest population size (49.05%). Cluster analysis based on percentage frequency of virulence of *P. graminis* race groups in different locations showed that in the 2016/2017 and 2017/2018 growing seasons, two main clusters were formed. Lines with *Sr* 24, *Sr* 38 and *Sr* 31 genes were showed the highest gene efficacy, while the other genes showed different reactions against the tested pathotypes.

**Keywords:** Wheat, *P. graminis*, Race identification, Virulence frequency, Geographical distribution, Cluster analysis, Phenotypic diversity.

### Introduction

Wheat rusts pose a potential threat to worldwide wheat production owing to their wide distribution, their capacity to form new races (pathotypes), and their ability to move long distances (Saari & Prescott, 1985; Kolmer, 2005). Rust diseases of wheat are still the most dangerous biotic stress that threatens wheat production in Egypt and many wheat-growing areas in the world (Kolmer, 2005; Wellings, 2011; Singh et al., 2011) and this is mainly due to the appearance of aggressive races of the pathogen (Singh et al., 2005). Wheat stem rust fungus could affect the entire wheat crop, especially during the early growth stages

leading to the blocking of the vascular system hence stunting and lodging of weak stalks eventually causing yield losses of even 100% due to shriveled grain and damaged tillers (Boukhatem et al., 2002; Kokhmetova et al., 2011).

Stem rust caused by *P. graminis* f. sp. *tritici* Eriks. & Henn. has been the most destructive disease of wheat (*Triticum aestivum* L.) under favorable conditions. Stem rust is also known as black rust due to the production of shiny black teliospores that produced at the end of the growing season or under unfavorable conditions (Singh et al., 2002).

<sup>#</sup>Corresponding author email: mohamedabo2002@yahoo.com

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Grain yield losses due to stem rust may reach up to 100% on the susceptible wheat cultivars under favorable environmental conditions (Roelfs, 1985). In the experimental field in Egypt, yield loss due to stem rust infection is 8.21% in the wheat cultivar Misr 1 (Ashmawy et al., 2013). Grain yield loss due to stem is usually high when the disease occurred early before the grain is completely formed, but yield losses are generally depending mainly on the dominant stem rust races, the resistance level of the cultivar grown, the growth stage when the initial infection occurred and the weather conditions (Luig, 1985). Hasan et al. (2016) and Gebrel et al. (2018) reported that wheat rust diseases have affected grain yield components of some Egyptian wheat cultivars and also observed a negative correlation between disease severity and grain yield.

Many control methods have been used to minimize the losses due to stem rust, but the use of resistant varieties is found to be the most economical, efficient, and farmer-friendly strategy (Abraham et al., 2018). At present, 82 stem rust resistance genes (*Sr*'s) have been described (McIntosh et al., 2017). The stem rust pathogen produces a large number of physiological races, which can be wind dispersal for long distances and infect wheat cultivars under favorable environmental conditions (Hei et al., 2018). The high virulence diversity and evolution rate of the pathogen make a considerable proportion of wheat germplasm at risk (Belayneh & Emebet, 2005). This fact, together with the ability to change genetically, is an opportunity for the force for created new races with increased aggressiveness on resistant wheat cultivars.

In recent years, variable races of stem rust pathogen have been identified in wheat production areas in different continents (Singh et al., 2008). In 1998, a new pathotype (Ug99) of *P. graminis* was identified in Uganda, which was virulent to the two stem rust resistance genes; *Sr* 31 and *Sr* 38 (Pretorius et al., 2000). This phenotype designated as TTKSK using the North American Stem Rust Nomenclature System (Jin et al., 2008). This pathotype was virulent to stem rust resistance genes; *Sr* 5, *Sr* 6, *Sr* 7b, *Sr* 8a, *Sr* 8b, *Sr* 9b, *Sr* 9e, *Sr* 9g, *Sr* 11, *Sr* 15, *Sr* 17, *Sr* 30, *Sr* 31 and *Sr* 38 (Jin et al., 2008). To date, 13 new variants (lineage) for Ug99 were virulent to stem rust resistance genes; *Sr* 24, *Sr* 36, *Sr* 9h, and *Sr* Tmp (Pretorius et al., 2012; Rouse et al., 2014). During the 2013/14

growing season, three variants of Ug99 pathotype, i.e., TTKST, TTKTK, and TTKSK, were detected in Egypt (Patpour et al., 2014).

Rust diseases survey is conducted in many wheat-growing areas in the world to monitor the new virulence phenotypes which provide information for the development of an early warning system and determine virulence shifts in a population. The survey provides essential information not only to determine the direction of the breeding program but also to detect new virulent phenotypes and the changes. This information helps to detect new race before build inoculums. Successful control for rust diseases requires understanding the virulent races present in the population and the impact of the use of resistant varieties on the frequency of pathotypes within a country. Thus, it is very important to consider the amount of diversity for virulence within the pathogen population and the sources of primary inoculum (McVey et al., 2004).

The objectives of this study were to identify and characterize stem rust races in Egypt in 2016/17 and 2017/18 growing seasons. Also, to study the geographical distribution of the identified pathotypes in six Egyptian governorates. Moreover, to estimate the phenotypic diversity between wheat stem rust pathogen populations in different locations in Egypt.

## **Materials and Methods**

### *Sample's collection and storage*

In 2015/2016 and 2016/2017, a total of 144 of infected stem rust samples (104 in 2015/16 and 40 in 2016/17) were collected from six locations of Egyptian wheat rust trap nurseries (EWRTN) experiment, i.e. Beheira, Kafr-Elsheikh, Sharqiya, Minufiya, Bani Sweif and Sohag. Stem rust samples were cut into small pieces of 5 to 10cm in length and placed in paper bags after the leaf sheath was separated from the stem to keep stem dry. This method helps the samples reduce moisture so the spores will not germinate before using it in the greenhouse. The collected samples in paper bags were labeled with the name of variety and district data and date of collection. Also, samples were kept at room temperature (18 to 24°C) overnight to dry their moisture content. Samples were kept in paper pages (8 x 15cm) in desiccators and kept in the refrigerator at 2 to 5°C until further use in the isolation process.

*Isolation, purification, and multiplication of single pustule isolates*

Five to ten seeds of the susceptible wheat cultivar; Morocco were planted into 10cm diameter plastic pots containing soil and peat moss in a 1:1 (v:v) ratio. When the first leaf fully emerged in seven days old seedlings, leaves were rubbed gently between moist fingers with tap water. Then infected stem samples were scraped using a sterile spatula and transferred to these seedlings and moistened with fine droplets of distilled water to form a film of free water, which is essential to initiate spore germination and establishment of infection. Finally, inoculated seedlings were incubated in dew chambers in darkness for 18hrs at 18 to 22°C and 98 to 100% relative humidity. Then inoculated plants were transferred to greenhouse benches where conditions were regulated at 12hrs photoperiod at a temperature of 18 to 25°C and relative humidity (RH) 60 to 70% (Stubbs et al., 1986). Two weeks later, after the pustule's rupture, three single pustules were isolated separately from each sample for spores multiplication on the highly

susceptible variety, Morocco to obtain enough urediniospores.

*Race identification and characterization*

The North American Stem Rust Differential Set (Roelfs & Martens, 1988; Jin et al., 2008) was used for race identification which included 20 wheat stem rust differentials lines with known stem rust resistance genes into five subsets; (1) *Sr* 5, *Sr* 21, *Sr* 9e, *Sr* 7b (2) *Sr* 11, *Sr* 6, *Sr* 8a, *Sr* 9g (3) *Sr* 36, *Sr* 9b, *Sr* 30, *Sr* 17 (4) *Sr* 9a, *Sr* 9d, *Sr* 10, *Sr* Tmp (5) *Sr* 24, *Sr* 31, *Sr* 38, *Sr* McN (Table 1). Five seeds for each of the above 20 differential lines were grown in 6 cm diameter plastic pots, each with seeds of four lines, planted in the corners of each pot in clockwise order. After seven-days, seedlings were inoculated with the previously isolated single pustule isolates of *P. graminis* f. sp. *tritici*, by shaking. The inoculated seedlings were incubated in the humid chamber overnight (100% RH), as described above. The inoculated seedlings were also transferred onto the greenhouse benches.

**TABLE 1. Five characters codes of *Puccinia graminis* (Pg) depend on high (H) and low (L) infection types (IT) on 20 wheat monogenic lines (5 sets).**

Pg-code <sup>a</sup>	Infection type <sup>b</sup> on monogenic lines with indicated Sr gene				
	Host set 1:	5	21	9e	7b
	Host set 2:	11	6	8a	9g
	Host set 3:	36	9b	30	17
	Host set 4:	9a	9d	10	Tmp
	Host set 5:	24	31	38	McN
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

<sup>a</sup> The race code consists of the letter designation for the pattern of ITs for the *P. graminis* f. sp. *tritici* isolates on a differential set 1 followed by set 2, then set 3, set 4 and set 5.

<sup>b</sup> L= Low infection type (avirulent isolate); H= high infection type (virulent isolate).

### Disease assessment

Stem rust infection types (IT) were scored 14 days after inoculation using the 0-4 scale of Roelfs et al. (1992). Entries that showed low infection types (L), i.e., scores= 0, 0; , 1 and 2 were considered as host resistant and avirulent isolates, while those showed high infection types (H), i.e., scores= 3 and 4 were recorded as the susceptible lines and virulent isolates. Every single isolate was assigned five letters based on high or low infection types to the differential lines (Roelfs & Martens, 1988; Jin et al., 2008).

### Determination of virulence frequency (%)

Percentage of virulence frequency was calculated for each of an identified race in the study as a number of virulent isolates to the total number of the tested isolates, according to the following equation:

$$\text{Virulence frequency (\%)} = \frac{\text{No. of virulent isolates}}{\text{Total number of isolates}} \times 100$$

### Race diversity measurements

The number and frequency of races within populations of *P. graminis* collected from six governorates in Egypt were compared and used to measure the diversity of each population within the different regions. To assess the diversity of *P. graminis* races within each population (region), three indexes, i.e. Shannon index ( $H_{SH}$ ), Gleason index ( $H_G$ ) and Simpson index ( $H_S$ ) (Groth & Roelfs, 1987) were estimated as follows:

#### Shannon index

It was used to determine the similarities of the frequencies of the different pathotypes in a set of isolates by the following formula:

$$H_{SH} = -\sum (P_i \ln P_i)$$

where:  $P_i$  = The frequency of the  $i$ th pathotype in the set of isolates.

#### Gleason index

It was used to detect the number of distinct pathotypes present, indicating the richness aspect of diversity and calculated by the following formula:

$$H_G = (n-1)/\ln(N)$$

where:  $n$  is the number of pathotypes, and  $N$  is the number of isolates in the sample population.

#### Simpson index

It was another popular diversity index for plant pathogens to determine the number of pathotypes

and evenness of their distribution and was calculated by the following formula:

$$H_S = 1 - \sum [n_i(n_i - 1)/N(N-1)]$$

where:  $n_i$  = The number of isolates of the  $i$ th pathotype and  $N$  = the sample size.

### Cluster analysis

A similarity matrix of all races identified in the governorates under study was used to construct a dendrogram, using the unweighted pair group method with arithmetic means clustering method in numerical taxonomy system (NTSYS-pc version 2.1) and based on the simple matching coefficient according to Rohlf (2000).

## Results

A total of 144 samples were collected during the two growing seasons (104 samples in the 2015/16 growing season and 40 samples in 2016/17 growing season). In 2015/16, the 104 samples produced 126 single isolates. The highest number of the collected samples was obtained from Kafr-Elsheikh and Sharqiya (each with 34 samples), while the lowest number of these samples was obtained from Minufiya and Bani Sweif (each with only one sample). The highest number of single isolates of stem sample rust pathogen was obtained from the samples collected from Kafr-Elsheikh and Sharqiya, which produced 39 and 38 isolates, respectively. While, the lowest number of these isolates was obtained from Bani Sweif and Minufiya, which produced 2 and 5 isolates, respectively (Table 2).

A total of 40 samples were collected in the 2016/17 growing season from six Egyptian governorates. These samples produced 64 single isolates. The highest numbers of the collected samples were obtained from Sharqiya; 25 samples. On the other hand, the lowest samples collected from from Minufiya and Sohag (each with one sample). Samples collected from Sharqiya was produced the highest number of single isolates; 32 (Table 2).

During the two growing seasons of the study, the highest number of the collected samples were from Sharqiya and Kafr-Elsheikh; 59 and 37 samples, respectively, which also showed the highest number of stem rust isolates; 70 and 47, respectively. While, the lowest number of isolates was obtained from the samples of Minufiya; 7 isolates (Table 2).

**TABLE 2. Number of stem rust samples and isolates collected from different wheat-growing locations from Egypt during the 2015/2016 and 2016/2017 growing seasons.**

Location	Growing season/ number of samples and isolates					
	2015/2016		2016/2017		Total number	
	No of samples	No. of isolates	No of samples	No. of isolates	No of samples	No. of isolates
Beheira	16	19	5	11	21	30
Kafr-Elsheikh	34	39	3	8	37	47
Sharqiya	34	38	25	32	59	70
Minufiya	1	5	1	2	2	7
Bani Sweif	1	2	5	9	6	11
Sohag	18	23	1	2	19	25
<b>Total</b>	<b>104</b>	<b>126</b>	<b>40</b>	<b>64</b>	<b>144</b>	<b>190</b>

#### *Virulence structure and identified stem rust pathotypes*

A total of 122 pathotypes of *P. graminis* f. sp. *tritici* were identified in Egypt during 2016/17 and 2017/18 growing seasons (Table 3). Stem rust races TKTTC and TTTTC are the most common races (18.25 and 17.46%, respectively), followed by the two races; TKTSC and TKTQC (3.97% and 2.38%, respectively). On the other hand, the seven stem rust races, i.e., PKRTC, TKRSC, TKRTC, TQTTC, TTRSC, TTRTC, and TTSTC, showed relatively low frequency, each with only 1.59% frequency (Table 3).

In 2017/18, a total of 53 races of *P. graminis* f. sp. *tritici* were identified from 64 isolates. The three stem rust races, PKSTC, BBBBC, and PKSTH, showed high frequency; 6.25%, 4.69%, and 4.69%, respectively, followed by the four races FKSSC, LBBBC, LBQBC and PFSTH as they showed by 3.12% for each. On the other hand, 46 out of 53 identified races were represented by only a single isolate which found at the lowest frequency (each with only 1.56% frequency) (Table 3).

Moreover, only one race, TKTTC was common and has been detected in the two growing seasons of the study, which showed high frequency; 18.25% in 2016/17 and 1.56% in 2017/18. Meanwhile, some of stem rust races under study were detected in the only first growing season, and not found in the second one, but others have been detected for the first time in the second growing season (Table 3).

#### *Geographical distribution:*

In 2016/17, a total of 24 pathotypes were

identified in Kafr-Elsheikh, which consider the largest population size, as represented by 34.28% frequency. Followed by the three governorates; Sharqiya, Beheira, and Sohag, which showed 27.14, 24.28, and 21.43% frequency, respectively of the whole population. While, each of the two governorates Bani Sweif and Minufiya, showed a small population size (2.86 and 7.14%, respectively).

In 2017/18, the Sharqiya governorate included the highest number of the identified pathotypes and considered to be the largest population size rather than the other pathogen population studied represented by 49.05% frequency. Followed by the three governorates; Beheira, Bani Sweif and Kafr-Elsheikh, which showed frequency 18.87, 13.21 and 11.32%, respectively while the lowest numbers of the identified races were found in Minufiya and Sohag (each with 3.77% frequency) (Table 4).

#### *The similarity of identified race groups in different locations*

To display the relationships between stem rust populations in different geographic wheat locations and similarity based on the percentage frequency of virulence race groups in six areas were illustrated in Fig. 1. As indicated in the cluster analysis of similarities, the studied locations in the 2016/2017 growing season formed two main clusters. The first cluster included five locations from a total of six locations. This cluster divided into two sub-clusters; the first contained only Beheira, and the second included Sharqiya, Minufiya, Bani Sweif, and Sohag. Moreover, the second cluster included only Kafr-Elsheikh (Fig. 1A).

**TABLE 3. Frequency (%) and races number of *Puccinia graminis* f. sp. *tritici* in Egypt during 2016/2017, and 2017/2018 growing seasons.**

Race	Races number and frequency (%)				Race	Races number and frequency (%)			
	2016/17		2017/18			2016/17		2017/18	
	No. of races	Frequency (%)	No. of races	Frequency (%)		No. of races	Frequency (%)	No. of races	Frequency (%)
CFCQC	1	0.79	-	-	KTTTC	1	0.79	-	-
BBBBC	-	-	3	4.69	LBBBC	-	-	2	3.12
BBBGC	-	-	1	1.56	LBBMC	-	-	1	1.56
BBBQC	-	-	1	1.56	LBJLR	-	-	1	1.56
BBGCC	-	-	1	1.56	LBQBC	-	-	2	3.12
BBGNC	-	-	1	1.56	LBTLL	-	-	1	1.56
BBJBC	-	-	1	1.56	LFRRC	-	-	1	1.56
BBSBC	-	-	1	1.56	LHRTC	-	-	1	1.56
BBSCC	-	-	1	1.56	LHSSC	-	-	1	1.56
BCDCC	-	-	1	1.56	LKQTC	1	0.79	-	-
BCSJC	-	-	1	1.56	LKSMC	-	-	1	1.56
BFQTC	-	-	1	1.56	LMBKC	-	-	1	1.56
BGBJC	-	-	1	1.56	MBBTC	-	-	1	1.56
BGGLC	-	-	1	1.56	MFNTC	-	-	1	1.56
BGLTC	-	-	1	1.56	MTQMC	1	0.79	-	-
BJTKC	-	-	1	1.56	NCSKC	-	-	1	1.56
BKQQC	-	-	1	1.56	NHMSC	-	-	1	1.56
CGBTC	-	-	1	1.56	NHTQC	-	-	1	1.56
DCQDH	-	-	1	1.56	NJQPC	-	-	1	1.56
DTTQC	1	0.79	-	-	PFJTC	-	-	1	1.56
FCQTC	1	0.79	-	-	PFSTH	-	-	2	3.12
FFGMC	-	-	1	1.56	PJLCC	-	-	1	1.56
FKSSC	-	-	2	3.12	PJQJC	1	0.79	-	-
FKSTC	-	-	1	1.56	PKHTC	1	0.79	-	-
FTCJC	1	0.79	-	-	PKKTC	-	-	1	1.56
FTTTC	1	0.79	-	-	PKMTC	1	0.79	-	-
GFTJC	-	-	1	1.56	PKRTC	2	1.59	-	-
HHKPC	1	0.79	-	-	PKSSH	-	-	1	1.56
HHRSC	1	0.79	-	-	PKSTC	-	-	4	6.25
HKSKC	1	0.79	-	-	PKSTH	-	-	3	4.69
JJHQC	1	0.79	-	-	PQBTC	-	-	1	1.56
JKTTC	1	0.79	-	-	PQQLC	1	0.79	-	-
JTTTC	1	0.79	-	-	PTQBC	-	-	1	1.56
KHQKC	1	0.79	-	-	PTQMC	1	0.79	-	-
KJPTH	1	0.79	-	-	PTTTF	1	0.79	-	-
KKTKC	1	0.79	-	-	QBGGC	-	-	1	1.56
KKTSC	1	0.79	-	-	QHTTC	1	0.79	-	-
KKTTC	1	0.79	-	-	QKGLC	1	0.79	-	-
KPGLC	1	0.79	-	-	QKTTB	1	0.79	-	-
KTRPC	1	0.79	-	-	QTQTM	1	0.79	-	-
RHGSC	1	0.79	-	-	TKRSC	2	1.59	-	-
RJRTC	1	0.79	-	-	TKRTC	2	1.59	-	-

TABLE 3. Cont.

Race	Races number and frequency (%)				Race	Races number and frequency (%)			
	2016/17		2017/18			2016/17		2017/18	
	No. of races	Frequency (%)	No. of races	Frequency (%)		No. of races	Frequency (%)	No. of races	Frequency (%)
RKTFC	1	0.79	-	-	TKSSC	-	-	1	1.56
RKTTC	1	0.79	-	-	TKSTC	1	0.79	-	-
RQLRC	-	-	1	1.56	TKTMK	-	-	1	1.56
RTTTC	1	0.79	-	-	TKTQC	3	2.38	-	-
SGMRC	1	0.79	-	-	TKTSC	5	3.97	-	-
SJRFC	1	0.79	-	-	TKTTC	23	18.25	1	1.56
SKJTC	1	0.79	-	-	TNTTC	1	0.79	-	-
SKQBC	1	0.79	-	-	TPTTC	1	0.79	-	-
SKSTC	1	0.79	-	-	TQTTTC	2	1.59	-	-
SLKDC	1	0.79	-	-	TRRTC	1	0.79	-	-
SPSSC	1	0.79	-	-	TRTTC	1	0.79	-	-
STSTC	1	0.79	-	-	TSSTC	1	0.79	-	-
TFTSC	1	0.79	-	-	TTDTC	1	0.79	-	-
TJTMC	-	-	1	1.56	TTMNC	1	0.79	-	-
TKGKC	1	0.79	-	-	TTRSC	2	1.59	-	-
TKJSC	1	0.79	-	-	TTRTC	2	1.59	-	-
TKJTC	1	0.79	-	-	TTSTC	2	1.59	-	-
TKMRC	1	0.79	-	-	TTTSH	1	0.79	-	-
TKQTC	1	0.79	-	-	TTTTTC	22	17.46	-	-
Total					122	<sup>b</sup> 126	100	64	100

<sup>a</sup>Races designated with five characters code for the five sets of wheat monogenic lines (*Sr*s) (Roelfs & Martens, 1988; Jin et al., 2008).

<sup>b</sup>Total number of identified pathotypes during the two growing seasons of the study= 123 (70 pathotypes during 2016/17 and 53 pathotypes during 2017/18 and the number here is 122 due to one pathotype was detected in the two seasons of the study).

In the 2017/2018 growing season, the similarity of the studied locations divided into two main groups. The first group included five locations. This cluster divided into two sub-clusters; the first contained only Beheira, and the second included Kafr-Elsheikh, Minufiya, Sohag, and Bani Sweif. On the other hand, the second cluster included only Sharqiya (Fig. 1B).

#### Virulence frequency (%)

Out of the tested pathotypes, only seven pathotypes were common during the 2016/17 and 2017/18 growing seasons, as they found in at least three isolates within populations and showed the most frequency in their populations (Table 5). Race TKTTC was the most frequent (18.25 % frequency) in 2016/17, but race PKSTC was the most frequent (6.25 % frequency) in 2017/18. Race TTTTC was the most aggressive race, where it found to be virulent to 17 monogenic lines

(*Sr*,s) and showed the highest virulence frequency (85.00%), followed by the five races; TKTTC, PKSTH, TKTSC, PKSTC, and TKTQC which showed virulence frequency; 80.00, 75.00, 75.00, 70.00 and 70.00 %, respectively. While pathotype BBBBC showed the lowest virulence frequency (5.00%).

#### Effectiveness of stem rust resistance genes at the seedling stage

In 2016/17 growing seasons, the three stem rust resistance genes; *Sr* 24, *Sr* 38, and *Sr* 31 were the most effective at the seedling stage, which showed 99.21, 99.21, and 98.41% efficacy, respectively against all of the 126 tested isolates. While, the stem rust monogenic lines, *Sr* McN, *Sr* 6, and *Sr* 9b were showed the lowest gene efficacy (%); 0.00, 2.38, 3.17, respectively. On the other hand, the other tested monogenic lines showed gene efficacy (%) ranged from 3.97% to 57.14% (Table 6).

**TABLE 4. Geographical distribution and frequency (%) of *Puccinia graminis* f. sp. *tritici* isolates identified in six Egyptian locations during the 2016/2017 and 2017/2018 growing seasons.**

No.	Governorate	Growing season/ identified isolates and their frequency (%)			
		2016/17		2017/18	
		Identified isolates & frequency (%)	Total No. of races	Identified isolates & frequency (%)	Total No. of races
1	Beheira	CFCQC (0.79), DTTQC (0.79), FTTTC (0.79), JJHQC (0.79), PKHTC (0.79), PQQLC (0.79), PTQMC (0.79), RKTTC (0.79), SJRFC (0.79), SPSSC (0.79), TFTSC (0.79), TKGKC (0.79), TKSTC (0.79), TKTSC (0.79), TKTTC (2.38), TNTTC (0.79), TTRSC (0.79)	17 (24.28%)	BGBJC (1.56), BGLTC (1.56), FFGMC (1.56), FKSTC (1.56), LHRTC (1.56), LMBKC (1.56), PFJTC (1.56), PKSTC (3.12), TJTMC (1.56), TKTMK (1.56)	10 (18.87%)
2	Kafrelsheikh	FTCJC (0.79), KKTTC (0.79), KPGLC (0.79), KTTTC (0.79), LKQTC (0.79), MTQMC (0.79), PKMTC (0.79), QKGLC (0.79), QTQTM (0.79), SKJTC (0.79), SKQBC (0.79), SLKDC (0.79), TKJSC (0.79), TKJTC (0.79), TKMRC (0.79), TKRSC (1.59), TKTSC (1.59), TKTTC (7.94), TQTTC (0.79), TTDTC (0.79), TTMNC (0.79), TTTTC (5.56)	24 (34.28%)	BJTKC (1.56), LBBBC (3.12), LBJLR (1.56), LHSSC (1.56), QBGGC (1.56), RQLRC (1.56)	6 (11.32%)
3	Sharqiya	HHKPC (0.79), JKTTC (0.79), JTTC (0.79), KKTSC (0.79), PKRTC (0.79), QHTTC (0.79), QKTTB (0.79), RHGSC (0.79), RJRTC (0.79), RTTTC (0.79), TKRTC (0.79), TKTQC (0.79), TKTSC (1.59), TKTTC (7.94), TPTTC (0.79), TSSTC (0.79), TTSTC (0.79), TTTSH (0.79), TTTTC (7.94)	19 (27.14%)	BBBQC (1.56), BBJBC (1.56), BCDCC (1.56), BCSJC (1.56), BFQTC (1.56), BGGLC (1.56), BKQQC (1.56), CGBTC (1.56), DCQDH (1.56), FKSSC (3.12), GFTJC (1.56), LBBMC (1.56), LBQBC (3.12), LFRRC (1.56), LKSMC (1.56), NCSKC (1.56), NHTQC (1.56), NJQPC (1.56), PFSTH (3.12), PJLCC (1.56), PKKTC (1.56), PKSSH (1.56), PKSTC (3.12), PKSTH (4.69), TKSSC (1.56), TKTTC (1.56)	26 (49.05%)
4	Minufiya	HKSKC (0.79), KJPTH (0.79), KTRPC (0.79), PKRTC (0.79), RKTFC (0.79),	5 (7.14%)	MBBTC (1.56), MFNTC (1.56)	2 (3.77%)
5	Bani Sweif	HHRSC (0.79), KKTKC (0.79)	2 (2.86%)	BBBBC (4.69), BBBGC (1.56), BBGCC (1.56), BBGNC (1.56), BBSBC (1.56), BBSCC (1.56), NHMSC (1.56)	7 (13.21%)
6	Sohag	FCQTC (0.79), KHQKC (0.79), PJQJC (0.79), PTTTF (0.79), SGMRC (0.79), SKSTC (0.79), STSTC (0.79), TKQTC (0.79), TKTQC (1.59), TQTTC (0.79), TRRTC (0.79), TRTTC (0.79), TTRTC (1.59), TTSTC (0.79), TTTTC (3.97)	15 (21.43%)	PQBTC (1.56), PTQBC (1.56)	2 (3.77%)
<b>Total</b>			<b>82*</b>		<b>53*</b>

\* Total No. of pathotypes in 2016/17 is 70 and in 2017/18 is 53, and the differences between these numbers are due to the presence of some pathotypes in more than one location.

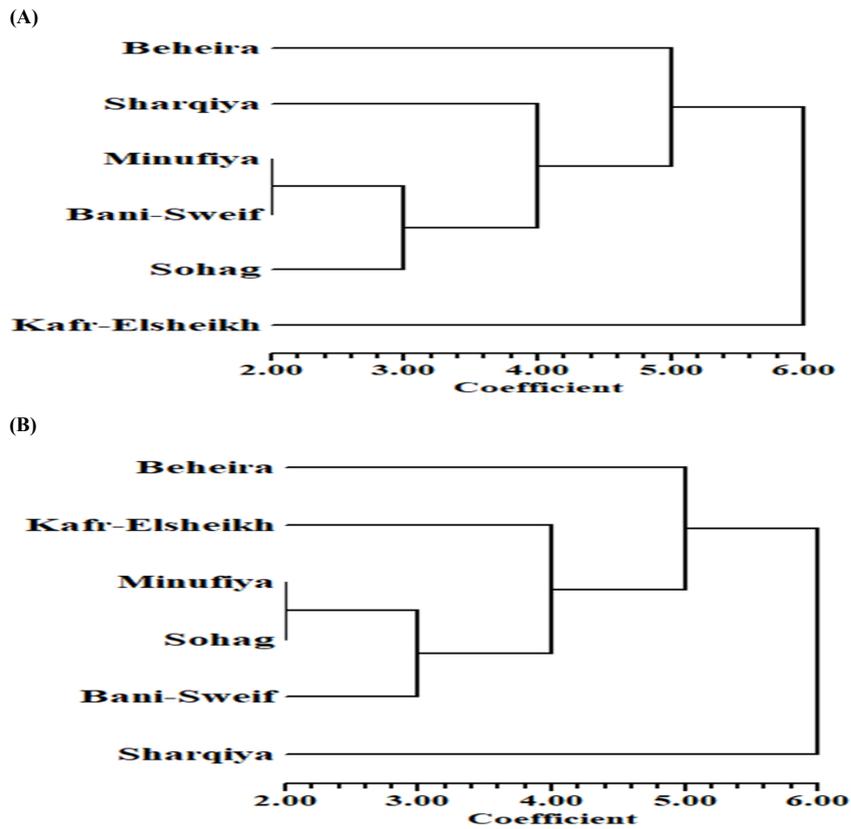


Fig. 1. Dendrogram of similarity and distribution of *P. graminis* isolates in 6 locations of Egypt during 2016/2017 (A) and 2017/18 (B) growing seasons.

In 2017/18 growing seasons, the five stem rust resistance genes; *Sr* 24, *Sr* 38, *Sr* 11, *Sr* 21 and *Sr* 31 were the most effective at the seedling stage, which showed 100.00, 98.44, 93.75, 90.63 and 90.63% efficacy, respectively against all of the 64 tested isolates. While the three stem rust monogenic lines, *Sr* McN, *Sr* 9b, and *Sr* 36 were showed the lowest gene efficacy (%); 0.00, 25.00, and 34.38%, respectively. On the other hand, the other tested monogenic lines showed gene efficacy (%) ranged from 37.50% to 78.13% (Table 6).

#### The diversity between stem rust populations

Three indexes of diversity, *i.e.*, Shannon ( $H_{SH}$ ), Gleason ( $H_G$ ), and Simpson ( $H_S$ ), were mainly used to measure phenotypic variation between different populations under study. These indexes were calculated for the six populations during the two growing seasons; 2016/17 and 2017/18 (Table 7 and Fig. 2).

In the 2016/17 growing season, the obtained values of the Shannon index ranged from 0.076 to 0.998, while Gleason index values ranged

from 0.200 to 0.895. Also, the Simpson index values ranged from 0.667 to 0.982. The highest phenotypic diversity of the Shannon index was observed in the four governorates; Kafr-Elsheikh, Sharqiya, Sohag, and Beheira which showed values; 0.998, 0.979, 0.719 and 0.701, respectively while the lowest values were found in Bani Sweif and Minufiya populations; 0.076 and 0.191, respectively (Table 7 and Fig. 2). The Gleason index values of the four governorates, Sharqiya, Kafr-Elsheikh, Beheira, and Sohag, were high, as it was 0.895, 0.872, 0.842, and 0.783, respectively. While, the two pathogen populations of Minufiya and Bani Sweif governorates showed the lowest values of the Gleason index; 0.200 and 0.500, respectively. On the other hand, stem rust populations of the five governorates; Beheira, Sohag, Kafr-Elsheikh, Minufiya and Sharqiya, showed the highest values of Simpson index, as they were 0.982, 0.952, 0.906, 0.900 and 0.870, respectively while the pathogen population in the Bani Sweif governorate showed the lowest value of the Simpson index (with only 0.667) (Table 7 and Fig. 2).

**TABLE 5. Virulence formula, number of races, frequency within the population (%) and virulence frequency (%) of the most common *Puccinia graminis* f. sp. *tritici* races in Egypt during 2016/2017 and 2017/2018 growing seasons.**

No.	Races <sup>a</sup>	Wheat growing season /virulence formula, number of races, frequency (%) & virulence frequency (%) of races				No. of ineffective genes	Virulence frequency (%) <sup>b</sup>
		2016/17		2017/18			
		Virulence formula (ineffective genes)	No. of races	Frequency (%)	No. of races		
1	BBBBC <i>Sr</i> McN	-	-	3	4.69	1	5.00
2	PKSTC <i>Sr</i> 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 30, 36, Tmp, McN	-	-	4	6.25	14	70.00
3	PKSTH <i>Sr</i> 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 30, 31, 36, Tmp, McN	-	-	3	4.69	15	75.00
4	TKTQC <i>Sr</i> 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 30, 36, McN	3	2.38	-	-	14	70.00
5	TKTSC <i>Sr</i> 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 30, 36, McN	5	3.97	-	-	15	75.00
6	TKTTC <i>Sr</i> 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 17, 21, 30, 36, Tmp, McN	23	18.25	-	-	16	80.00
7	TTTTTC <i>Sr</i> 5, 6, 7b, 8a, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 36, Tmp, McN	22	17.46	-	-	17	85.00
<b>*Others (less than 3 isolates)</b>		<b>73</b>	<b>57.94</b>	<b>54</b>	<b>84.37</b>	-	-
<b>Total</b>		<b>126</b>	<b>100</b>	<b>64</b>	<b>100</b>	-	-

<sup>a</sup> Pathotypes that did not found of at least 3 isolates within-population were excluded from the table.

<sup>b</sup> Virulence frequency (%) calculated by dividing the number of ineffective genes for each pathotype to the total number of differential genes (20 genes) X 100.

In the 2017/18 growing season, Shannon index values ranged from 0.130 to 0.998, while Gleason index values ranged from 0.375 to 0.781. Also, the Simpson index values ranged from 0.667 to 0.992. The highest phenotypic diversity values of the Shannon index were found in the three governorates; Sharqiya, Beheira and Bani Sweif Beheira, which showed 0.998, 0.692 and 0.533, respectively. While, the lowest Shannon index values were found in the three governorates; Kafr-Elsheikh, Minufiya, and Sohag, i.e. 0.498, 0.130 and 0.130, respectively. Gleason index values in the four governorates; Sharqiya, Bani Sweif, Minufiya and Sohag, were high as they showed 0.781, 0.555, 0.500 and 0.500 values, respectively. While, the two pathogen populations in Beheira and Kafr-Elsheikh showed the lowest values; 0.454 and 0.375, respectively. Simpson index values of the populations; Sharqiya, Beheira, Kafr Elsheikh and Bani Sweif, were high as they showed

0.992, 0.982, 0.964, 0.700 values, respectively. While the two pathogen populations in the two governorates Minufiya and Sohag, showed the lowest values; each with 0.667 (Table 7 and Fig. 2).

Data in Table 8 revealed that the correlations of the four components of diversity, i.e. sample size collected for each region, number of isolates, number of races and standard deviation of race frequency to the three diversity indexes, i.e. Shannon, Gleason and Simpson over the two growing seasons of the study. Significant correlation of Shannon index was found between each of the sample size collected for each region, number of isolates, number of races and standard deviation of race frequency, indicating that this index was sensitive to sample size ( $r = 0.933$ ), number of isolates ( $r = 0.871$ ), number of races ( $r = 0.890$ ) and standard deviation of race frequency ( $r = 0.905$ ). While, no correlations

were present between the four components of diversity to the other two diversity indexes, i.e., Gleason and Simpson. So, It can conclude that

the Shannon index was the most important to calculate phenotypic diversity in *P. graminis* f. sp. *tritici* populations under Egyptian conditions.

**TABLE 6. Gene efficacy (%) of 20 stem rust resistance genes at the seedling stage during 2016/17 and 2017/2018 growing seasons.**

No.	Srs	2016/17			Gene efficacy (%)	2017/18			Gene efficacy (%)
		No. of pathotypes		Total		No. of pathotypes		Total	
		Avirulent	Virulent			Avirulent	Virulent		
1	5	16	110	126	12.70	24	40	64	37.50
2	21	11	115	126	8.73	58	6	64	90.63
3	9e	12	114	126	9.52	32	32	64	50.00
4	7b	13	113	126	10.32	32	32	64	50.00
5	11	72	54	126	57.14	60	4	64	93.75
6	6	3	123	126	2.38	26	38	64	40.63
7	8a	9	117	126	7.14	30	34	64	46.88
8	9g	10	116	126	7.94	28	36	64	43.75
9	36	11	115	126	8.73	22	42	64	34.38
10	9b	4	122	126	3.17	16	48	64	25.00
11	30	32	94	126	25.40	32	32	64	50.00
12	17	18	108	126	14.29	50	14	64	78.13
13	9a	5	121	126	3.97	24	40	64	37.50
14	9d	8	118	126	6.35	27	37	64	42.19
15	10	12	114	126	9.52	28	36	64	43.75
16	Tmp	27	99	126	21.43	27	37	64	42.19
17	24	125	1	126	99.21	64	0	64	100.00
18	31	124	2	126	98.41	58	6	64	90.63
19	38	125	1	126	99.21	63	1	64	98.44
20	McN	0	126	126	0.00	0	64	64	0.00

**TABLE 7. Phenotypic diversity of wheat stem rust races collected from six governorates in Egypt during 2016/2017 and 2017/2018 wheat growing seasons.**

Governorate	Growing season/ diversity index					
	2016/2017			2017/2018		
	Shannon	Gleason	Simpson	Shannon	Gleason	Simpson
Beheira	0.701	0.842	0.982	0.692	0.454	0.982
Kafrelsheikh	0.998	0.872	0.906	0.498	0.375	0.964
Sharqiya	0.979	0.895	0.870	0.998	0.781	0.992
Minufiya	0.191	0.200	0.900	0.130	0.500	0.667
Bani Sweif	0.076	0.500	0.667	0.533	0.555	0.700
Sohag	0.719	0.783	0.952	0.130	0.500	0.667

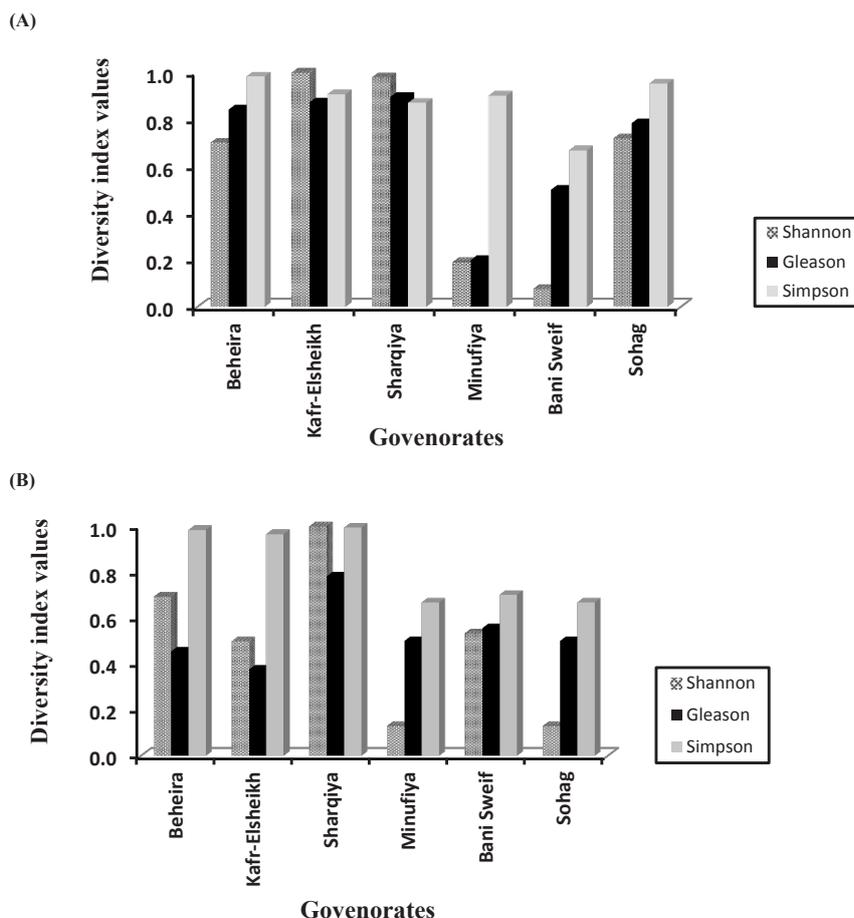


Fig. 2. Shannon, Gleason, and Simpson indexes of phenotypic diversity for six populations of *P. graminis* pathotypes in Egypt during (A) 2016/2017 and (B) 2017/2018 wheat growing seasons.

TABLE 8. Correlations between the independent components and the three indexes of diversity from six pathogen populations of *P. graminis* in Egypt during 2016/2017 and 2017/2018 wheat growing seasons.

Diversity index	Components of diversity			
	Sample size	No. of isolates	Race number	The standard deviation of race frequency
Shannon	0.933*	0.871*	0.890*	0.905*
Gleason	-0.450 <sup>ns</sup>	-0.392 <sup>ns</sup>	0.298 <sup>ns</sup>	-0.349 <sup>ns</sup>
Simpson	0.228 <sup>ns</sup>	0.184 <sup>ns</sup>	0.371 <sup>ns</sup>	0.266 <sup>ns</sup>

- \* Coefficients are significant at 0.05.

- ns= Non-significant.

## Discussion

Based on the results of the present investigations obtained for the two growing seasons, that is, 2016/2017 and 2017/2018 revealed that total of 122 physiologic races of *P. graminis* were identified among 190 collected

isolates. Also, the survey of the virulence pathotypes in some governorates in Egypt during two growing seasons showed that pathotypes TKTTC and TTTTC were the most common for 2016/2017. While, for 2017/2018, the most dominant and common races were PKSTC, BBBBC, and PKSTH. Similar results were

obtained by Olivera et al. (2010), who reported that races TRTTF and JRCQC were the most aggressive pathotypes in durum growing areas of Ethiopia. Races very similar to TRTTF have also been identified from collections originating in Yemen and Pakistan (Mirza et al., 2010); this group of races possesses virulence to resistance genes designated as *SrTmp* and *Sr1A.1R* (a gene present in wheat-rye translocation 1A.1R), which are effective against the Ug99 group of races. Identification of the TRTTF group of races in Africa, the Middle East, and Asia, with virulence to *Sr13*, *SrTmp*, and *Sr1A.1R*, reduces the focus in the utilization of these resistance genes in breeding efforts. In the present study, a total of 126 and 64 pathotypes were appeared in Egypt during the 2016/2017 and 2017/2018 growing seasons, respectively and this is likely due to the differences in the number of sampling between the two seasons. In the 2015/2016 growing season, the collected samples were 104 and 40 in 2016/2017. A higher number of identified races will likely be detected in 2017/2018 if the collected samples had been obtained from a larger number of sites.

The risk of a stem (black) rust epiphytotic has increased in recent years following the evolution in East Africa of race Ug99 (Singh et al., 2008; Joshi et al., 2011), which was able to overcome the widely used host resistance gene *Sr31* (Pretorius et al., 2000). Admassu et al. (2008) reported the effectiveness of *Sr36* in Ethiopia though virulence is detected in Kenya for the same gene (Jin et al., 2009). Resistance gene *Sr25* conferred a high level of resistance only in some genetic backgrounds, especially when the adult plant resistance gene *Sr2* was also present, e.g., in recently released Ug99-resistant varieties Misr 1 in Egypt and Muquawin 09 in Afghanistan. Gene *Sr26* was used successfully in Australia and remains effective despite its large-scale deployment in the 1970s, 1980s, and once again recently (McIntosh et al., 1995). Gene *Sr27* of rye origin has not been used in wheat improvement. Its deployment in triticale in Australia resulted in the rapid evolution of virulence (McIntosh et al., 1983). This gene has also become ineffective in South Africa. Strategically, this gene should be used for triticale improvement in areas where virulence is not known. Genes *Sr22* and *Sr35*, derived from *Triticum monococcum* are both highly effective against Ug99 and can be backcrossed to modern wheat. Virulence to *Sr35*

was identified in a laboratory culture in Australia (McIntosh et al. 1995). Although race Ug99 is a virulent to gene *Sr28*, numerous races virulent to this gene are known to occur worldwide. Genes *Sr33*, *Sr45*, and *Sr46*, derived from *Aegilops tauschii*, confer moderate resistance levels that are inadequate under stem rust pressure in screening nurseries in Kenya.

In 2016/17, Kafr Elsheikh was considered the largest population size, followed by three governorates; Sharqiya, Beheira, and Sohag, respectively, while the two governorates Bani Sweif and Minufiya showed a small population size. In 2017/18, the Sharqiya governorate considered being the largest population size followed by the three governorates; Beheira, Bani Sweif, and Kafr-Elsheikh, while the lowest numbers of identified races were found in Minufiya and Sohag.

The similarity between stem rust populations in different locations under study showed that in both growing seasons, there are significant differences between race groups frequency so the dendrogram of the tested race groups divided into two main clusters and this probably caused by the low number of collected field samples in the growing seasons.

Virulence against stem rust resistance genes showed that *Sr 24*, *Sr 38*, and *Sr 31* genes were the highest gene efficacy against race groups, while the other genes showed different reactions against the tested race groups depending on the aggressiveness of the tested race groups.

Stem rust has been a major problem historically in all of Africa, the Middle East, all of Asia (except Central Asia), Australia, New Zealand, Europe, and the Americas (both North and South) (Saari & Prescott, 1985). Various control options are available for combating stem rust. The use of resistant varieties has been the most effective means in low input agriculture (Roelfs et al., 1992). Until recently, stem rust of wheat has been successfully controlled through genetic resistance (Singh & Rajaram, 2002).

In our study, three diversity indexes, i.e., Shannon, Simpson and Gleason, were used to estimate phenotypic diversity of *P. graminis* populations in six different locations in Egypt. In general, Shannon, Gleason, and Simpson

indexes showed high values; this means that high diversity was found for the studied populations in most of the tested locations during the two growing seasons. The high diversity in different stem rust populations may be explained by the long-term and wide cultivation of the commercial Egyptian wheat cultivars that have a high stem rust resistance. Therefore, this makes a high selection pressure levels on *P. graminis* races. The three diversity indexes used in this study are little else in common and represent real choices in how best to characterize the diversity of pathogen populations. Thus, the selection of the suitable index must depend on the objectives of the study and the properties of the population(s) of the target pathogen to be characterized or compared with others. However, if populations of plant-pathogen are being characterized the kind of diversity indexes used will mainly depend upon the number of pathotypes, degree of dominance, and size of samples (Groth & Roelfs, 1987). The Gleason index is the most sensitive to the number of pathotypes; also, it is usually used if frequency data are not to be included. El-Orabey et al. (2018) studied the diversity in leaf rust races in Egypt using the three mentioned indexes. They found that the Shannon index is the most sensitive and suitable to estimate diversity in *P. graminis* races which showed high correlation with sample size ( $r = 0.933$ ), number of isolates ( $r = 0.871$ ), number of races ( $r = 0.890$ ) and standard deviation of race frequency ( $r = 0.905$ ). So, the use and application of more than one index to describe and estimate the phenotypic diversity of pathogen populations are desirable if several of the meanings of diversity are of interest. In particular, either the Simpson or Shannon index might be calculated for each of the two populations to assess the absolute magnitude of diversity (Groth & Roelfs, 1987).

### Conclusion

Survey of wheat stem rust pathotypes using stem rust differential monogenic lines is very important in discovering virulence pattern variation, geographical distribution of stem rust races, and how stem rust populations change in response to host selection. This action should be doing in all wheat growing seasons using rust survey and planting of rust trap nurseries in all Egyptian locations, including rust hot spot sites which will provide timely warning to wheat breeders about the change in virulence of *P. graminis* populations.

Thus, it will be very significant to avoid future stem rust epidemics and reduced annual losses of the commercial wheat cultivars grown in Egypt.

*Conflict of interest:* The authors declare that they have no conflict of interest.

*Ethical approval:* This article does not contain any studies with human participants or animals performed by any of the authors.

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### توصيف القدرة المرضية والتنوع داخل سلالات فطر بكسينيا جرامينيز على القمح في مصر

دعاء راغب النجار، وليد محمد العربي، محمد عبد الله جاد، جمالات عبد العزيز هرامس  
قسم بحوث أمراض القمح - معهد بحوث أمراض النباتات - مركز البحوث الزراعية - الجيزة - مصر.

يعتبر مرض صدأ الساق المتسبب عن فطر بكسينيا جرامينيز من الأمراض المدمرة للقمح في مصر والعالم. حصر عينات صدأ الساق في القمح وتعريف السلالات الفسيولوجية باستخدام عشرون جين فردى لصدأ الساق هام جداً في وصف التنوع في القدرة المرضية والتوزيع الجغرافي لسلالات صدأ الساق وكيفية تغيير رد فعلها تجاه العائل. يتم تحديد التنوع في عشائر المسبب المرضي باستخدام العينات التي تم جمعها من حقول القمح في مصر خلال الموسمين 2015/2016 و 2016/2017. أظهرت النتائج المتحصل عليها تباين كبير في سلالات الفطر والتي اختلفت من موسم لآخر. في هذه الدراسة، تم جمع 104 و 40 عينة من صدأ الساق في 2015/2016 و 2016/2017، على التوالي من مناطق زراعة القمح المختلفة في ستة محافظات في مصر، وهي البحيرة وكفر الشيخ والشرقية والمنوفية، بني سويف وسوهاج. تم عزل 70 و 53 سلالة فسيولوجية من العينات التي تم جمعها في السنوات السابقة والتي تم تعريفها في الموسمين 2016/2017 و 2017/2018، على التوالي. كانت أكثر السلالات تكرارية داخل عشيرة الفطر هي السلالة TKTTC (18.25%) و السلالة TTTTC (17.46%) وذلك في موسم 2016/2017. وكذلك PKSTC (6.25%) و BBBBC (4.69%) و PKSTH (4.69%) وذلك في موسم 2017/2018. في الموسم 2016/2017 تم تعريف 24 سلالة من الفطر في كفر الشيخ، والتي تعتبر أكبر العشائر حجماً (34.28%)، بينما في الموسم 2017/2018 تعتبر محافظة الشرقية أكبر العشائر حجماً (49.05%). أظهر التحليل التجميعي المعتمد على النسبة المئوية لتكرار القدرة المرضية لمجاميع الفطر *P. graminis* في المواقع المختلفة في الموسمين الزراعيين 2016/2017 و 2017/2018 تكون مجموعتين أساسيتين. أظهرت السلالات النباتية المحتوية على الجينات *Sr* 24، *Sr* 38، *Sr* 31 كفاءة عالية ضد سلالات الفطر بينما الجينات الأخرى أظهرت رد فعل متباين ضد السلالات المختبرة.